STUDIES ON CORRELATIONS BETWEEN URINE PARAMETERS AND FLUX VARIATIONS ON HUMAN URINE USING He-Ne LASER AND ENCIRCLED FLUX ANALYSIS SYSTEM

by

AZRUL NIZAM BIN ALIAS

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He	Helium	19-106
Ne	Neon	19-106
EFAS	Encircled Flux Analysis System	20-106
W	Watt	20-106
LDF	Laser Doppler flux	21
2D	2-dimensional	22,66-106
3D	3-dimensional	22,66-106
λ	Wavelength	23
SG	Specific Gravity	28-106

KAJIAN KORELASI ANTARA PARAMETER URIN DAN PERUBAHAN FLUKS PADA URIN MANUSIA MENGGUNAKAN LASER He-Ne DAN SISTEM ANALISIS FLUKS KETERBULATAN

ABSTRAK

Dalam kajian ini, hubungan antara parameter urin dikaji dengan mendapatkan corak dan ujian statistikal dengan menggunakan SigmaStat 3.1 dan variasi fluks menggunakan laser He-Ne 0.95 mW dan *Encircled Flux Analysis System* (EFAS). Data yang diperolehi daripada analisis statistikal menunjukkan hubungan selari dengan kajian lain. Keselarian ditunjukkan daripada hubungan yang diperolehi antara pH urin, umur, *specific gravity* (SG) urin, urin berprotein dan urin berglukosa. Sebaliknya, keputusan yang tidak konsisten dengan kajian lain adalah antara SG urin dan umur serta antara SG urin dan urin berprotein. Kajian parameter urin dengan variasi fluks mempamerkan corak yang penting. Puncak fluks dan jumlah fluks untuk lelaki menunjukkan corak peningkatan untuk pH urin dan SG sementara menunjukkan corak penurunan untuk wanita. Puncak fluks bagi lelaki dan jumlah fluks bagi perempuan menunjukkan peningkatan dengan umur sementara jumlah fluks bagi lelaki dan puncak fluks bagi perempuan menunjukkan penurunan. Corak 2D kontur dan 3D profail memberikan corak tersendiri berdasarkan jantina, kesihatan urin dan kumpulan umur. Kajian ini menunjukkan penggunaan laser He-Ne dan EFAS mempamerkan prospek masa depan yang baik dalam kajian urin. Parameter fluks seperti puncak fluks

dan jumlah fluks boleh menjadi parameter-parameter yang penting bagi analisis urin.

STUDIES ON CORRELATIONS BETWEEN URINE PARAMETERS AND FLUX VARIATIONS ON HUMAN URINE USING He-Ne LASER AND ENCIRCLED FLUX ANALYSIS SYSTEM

ABSTRACT

In this research, correlations between urine parameters is studied by finding patterns and statistical tests using SigmaStat 3.1 and flux variations using 0.95 mW He-Ne laser and Encircled Flux Analysis System (EFAS). Data obtained from statistical analysis shows correlations and consistencies with other researchers. These consistency are reflected from the correlations obtained between urine pH, age, urine specific gravity, urine protein and urine glucose. Conversely, the inconsistencies of the results are shown between urine specific gravity and age and also between urine specific gravity and urine protein. The studies on urine parameters by flux variations exhibits significant patterns. Flux peak and total flux for males show increasing pattern for urine pH and specific gravity while for females show decreasing pattern. Flux peak for males and total flux for females shows increasing pattern when aging while total flux for males and flux peak for females shows decreasing pattern. The pattern of 2D contour and 3D profile gives individual pattern according to gender, urine health and age groups. This research shows that using He-Ne laser and EFAS exhibited a good future prospect in urine research. Flux parameters such as flux peak and total flux, can become significant parameters for analysis of urine.

Chapter 1 : Introduction

1.1 Urine

Urine is a fluid which is continuosly formed in and excreted from the body. It supplies significant information with regard to many disorders and diseases [1]. Urine also has been referred to like a mirror, which reflects activities within the body [2]. It has been identified to presenting a biopsy of the kidney. It is the principal route of waste removal of products of metabolism from the body [3]. Disorders of the kidneys obviously modify the composition of the urine. But kidney disorders may also be complicate many other body processes. Urine studies may also reflect the situation when the function of the kidney is normal, but other parts of the body are out of synchronization [4].

The process of urine is formed has been of great interest in science and medicine [5]. A clear-cut concept of the mechanism of urine formation can be described, but there are a number of aspects which may be altered or expanded as additional insight is established. The understanding of mechanism of formed urine gives a basis for understanding many of the abnormalities of urine that are observed in disease.

The kidneys are bean-shaped organs and lie retroperitoneally on either side of the vertebral column. Normally, the 2 kidneys weight about 300 g, and thus constitute less than 0.5 % of the body weight [6]. The kidneys are quite close to the abdominal aorta and receive blood through large renal arteries. The cortex or outer portion of the kidney is reddish-brown in colour. This outer layer of the kidney dips down between adjacent pyramids towards the renal sinus [7].

The basic, microscopic functional unit of the kidney is the nephron. Figure 1.1 is a schematic diagram of a single nephron [8]. The understanding of the function of a single nephron provides a basis for understandings of the total functioning of the kidney. It is estimated that each human kidney contains approximately 1 to 1.25 million nephrons [9]. The glomerulus lies in the cortex or outer part of the kidney. The proximal convoluted tubule and the distal convoluted tubule are situated in the cortex of the kidney, whereas the descending loop of Henle and the ascending loop of Henle pass almost from the outer portion of the kidney to the center or medulla and back again. Finally, the collecting duct passes to the calyx or central portion of the kidney [10].

The afferent arteriole branches into several capillary loops within Bowman's capsule [11]. These loops are joined by several anastomoses and combine to form the efferent arteriole. The proximal convoluted tubule and distal convoluted tubule are lined with cuboidal cells. The cells are columnar in some portions of the tubule, quite flat in others [12].

A rich lymphatic network drains the cortex of the kidney, but there is no significant lymphatic circulation in the medulla or the papilla [13]. The kidney has an abundant nerve supply which is primarily sympathetic. The nerves have significant degree terminated in the afferent and the efferent arterioles. The sympathetic vasomotor nerves are primarily vasoconstrictor in function [14].

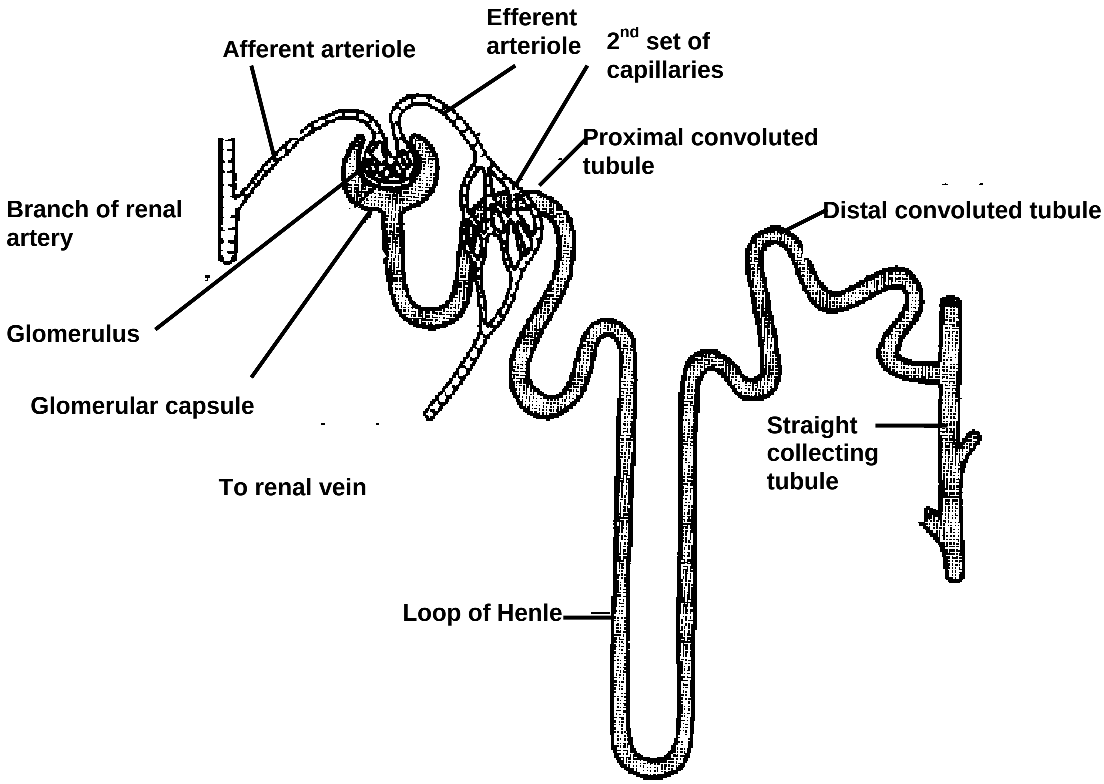

Figure 1.1. Schematic diagram of a single nephron [8]

The abdominal aorta considers a very great supplying blood to kidneys through the renal arteries [15]. The arteries subdivide and ultimately become arterioles which enter the glomeruli in the renal cortex. An ultrafiltration occurs within the capillary tufts of the glomeruli which are filtered the water and the solute low weight molecular from the blood [16]. In turn, the blood passes into the efferent arterioles which are closely approximated to the convoluted tubules. The formed ultrafiltrate in the glomerulus contents a quite composition of soluble solutes comparable to the blood which is derived. Therefore the large molecular weight constituents and cellular elements are both removed. When the ultrafiltrate passes into the renal tubule various constituents of the glomerular filtrate are selectively reabsorbed. Therefore, sodium chloride, water, amino acids, bicarbonate, glucose, uric acid and phosphate are reabsorbed in the proximal tubule as well as water is reabsorbed in the distal tubule, while in the collecting tubule, water, sodium chloride and urea are reabsorbed [17]. The process of reabsorption appears to be delicately regulated by endocrine mechanisms which involve adrenal cortical hormones and the antidiuretic hormone.

The proximal tubule absorbs large amounts of glucose, the fluid passing through this segment of the nephron retains the same osmolality as plasma [18]. The development on concentrated urines with high osmotic pressures is achieved due to the changing in osmolality in the distal tubule. Alternatively, the most of urine may lose its electrolytes and become quite dilute in this portion of the kidney. In the tubules, there is also an active process of tubular excretion which involves the excretion of numerous substances from the blood directly into the tubular urine [19]. The hydrogen ion is one of the substances excreted by the tubular cells, which promptly combines with ammonia or phosphate.

In order of magnitude, one might envision that there are approximately 1,000 litres of blood pass through the kidneys each day and approximately 100 litres of glomerular filtrate are formed during this period [20]. Then, most of this filtrate is reabsorbed, at that a final typical urine volume is 1 litre/day. From a functional standpoint, the process of urine formation provides the excretion of waste products and the regulation of body water, body pH, and body electrolytes [21].

Urine can be considered the most complex fluid in the body. It contains practically all of the constituents found in the blood. Although many substances found in the blood and in the urine, the concentration are different in the two body fluids. In many instances, the amount of a given urinary substance may far exceed the amount present in the blood. For example, urea has concentration with a normal blood about 20 mg/100 ml and with a normal urine about 3,000 mg/100 ml [22]. For other substances, the concentration present in urine may be very much less than in blood. Glucose is one of this type of substance, where the fasting blood concentration is about 90 mg/100 ml and the urine concentration is closer to 10 mg/100 ml [23].

The urine is a sparklingly clear fluid generally, which is yellow or amber [24]. It has a characteristic's odour which is not regarded as disagreeable by most persons. The urine may be turbid and yet be completely normal. Turbidity of urine specimens in healthy persons is due to precipitation of phosphate salts or uric acid in the bladder [25]. Such precipitation may occur due to changes in the acidity or alkalinity of the urine in the bladder. The odor of urine have modified in order to the kind of foods in the diet [26].

One of the essential functions of the kidney is excreted a waste materials and substances, which the body are not needed. Urine density is related primarily to an amount of excreted water, salt and urea [27]. The osmolality of the urine or other body fluids is an expression of the osmotic pressure. Osmolality and urine density are quite closely related, with the advantage that expressing values in osmolal units permits comparison of urine with blood and thus provides a slightly greater convenience in identifying renal activity [28].

Most of circumstances of human urine, the specific gravity is between 1.008 and 1.030, but the ingestion of large amounts of fluids decreases the specific gravity almost to 1.000 [29]. Someone does not ordinarily find the significant specific gravity in excess to 1.030, unless metabolites of certain drugs are being excreted or a large quantity of glucose or protein is existed. The ability of the kidney for excretion a dilute or concentrated urine is frequently measured by a dilution-concentration test [30]. Various procedures are used in such studies, and all are define how the kidney responds to a condition and situation wherever is need to excrete an increase water and deprivation of mild fluid [31].

Urine pH measurement is a part of most regular urinalysis [32]. The pH of urine is affected to a significance degree by the acidic or basic salts which are in the specimen. The mechanism of excretion acid or alkaline urine that the body can get rid of relatively large amounts of either acids and/or bases and maintain a constant homeostatic state [33]. Some chemical constituents of urine are mainly responsible for establishing the pH of any specific urine specimen [34]. These substances included sodium and potassium mono- and dihydrogen phosphates, sodium citrate, ammonium salts, sodium bicarbonate and carbonic acid. A great number of other substances have been normally made a smaller contribution to the final urinary pH. The majority of the substances are simply excreted from the blood into the urine by the kidney [35].

However, in the case of ammonium salts, the kidney actually converts a neutral urea into ammonia, providing a mechanism to excretion of acids from the body [36]. This conversion process is quite active in situations where the body tends to have an excess of acid. Correspondingly, if the body has an excess of base, the kidney synthesizes citrate in relatively large quantities, thus providing a mechanism for excretion of extra base [37]. Table 1.1 shows a comparison of the pH of urine, various body fluids and other material .

The pH of the urine of a healthy person reflects the acid-ash or alkaline-ash composition of the diet [38]. During the course of a day, the urine pH will ordinarily show rather rapid and large swings from acid to alkaline or vice versa. This can be recognized by a specimen being turbid at the voided time. This turbidity is most frequently caused by the fact that certain components which are quite soluble in an acid urine are precipitated when the specimen is made alkaline, as by the admixture in the bladder of an excess of alkaline urine [39]. Alternatively, certain substance that are soluble in an alkaline urine will precipitate if an excess acidity is established.

Table 1.1. The pH of urine compared with body fluids and other material [40]

Body fluids and other material	pH
Urine	4.8 – 8.5
Blood	7.4
Serum	7.4
Plasma	7.4
Saliva	6.75
Gastric juice	1.2 – 3.0
Pancreatic juice	8.7
Bile	7.5
Duodenal fluid	6.7
Jejunal fluid	6.5
Ileal fluid	7.1
Aqueous humor	7.2
Sweat	5.2
Milk	7.0
Semen	7.4
Tears	7.4
Interstitial fluid	7.4
Intracellular fluid – liver	6.9
Sea water	7.3
Tomato juice	4.3
Grapefruit juice	3.2
Cola soft drink	2.8
Lemon juice	2.3

The processing of adjustment of urinary pH by the kidney occurs in both the proximal tubule and the distal tubule, where a selective absorption of bicarbonate or secretion of ammonia occurs. According to current concepts of the process of urine formation, the glomerular filtrate has a pH, which is essentially the same for the blood. As the urine proceeds along the proximal tubule, the pH is lowered to 6.8 [41]. This occurs primarily as a result of selective reabsorption and tubular excretion. When a decrease in pH takes place, the active secretion of hydrogen ion occurs in the distal tubule and the pH may drop to values of less than 5. Ammonia is secreted in the distal tubule and due to the exist acid, it promptly combines to form an ammonium complex which is excreted in the urine.

During acidosis, ammonium excretion increases from 20 - 30 meq/day to more than 500 meq/day [42]. The cells of the tubule generate ammonia from a variety of amino acids, most notably glutamine [43]. The kidney loses its capacity to generate a significant degree of ammonia. Thus, the renal impairment patients lose their ability to excrete an acid load maximally. This tends to cause an acidosis in such patients. Addison's disease have also an impaired ammonia forming mechanism, which promptly disappears when corticosteroids are administered [44].

The extreme range of urine pH is change from pH 4.8 to pH 8.5 approximately [45]. In situations of extreme ketosis, the urine have a slightly lower pH, and in instances of severe infections of the kidney or bladder, the pH of the excretion urine excesses of pH 9 due to alkaline ammonium carbobate which is formed from urea. The physiological capability of tubular cells to selectively respond to very slight changes within the body (changes so slight they cannot at present be measured by available instrumentation) represents a most highly refined biological regulating mechanism [46].

At least three genetic disorders relate to loss of the ability of the distal tubular cells to make their contribution to body pH control [47]. In renal tubular acidosis, the kidney is incapable of forming a highly acid urine and accordingly when an excess of acid is presented to the body, the kidney is not able to contribute its usual control function. Therefore, the urine remains about neutral and severe acidosis results. In renal tubular alkalosis, tubular cells are unable to excrete an alkaline urine so that when alkali excesses are presented, alkolisis ensues. In the Fanconisyndrome, loss of renal acid excretory ability occurs with resulting acidosis [48].

If a large amount of water is ingested by a human, a corresponding diuresis or increase in urine excretion occurs. At this time, the pH of the urine tends to become relatively fixed at a value quite close to neutrality [49]. This phenomenon is indicated that the normal process of urine pH adjustment in the proximal and distal tubules does not have an opportunity to function effectively. The pH of the urine becomes quite close to the blood. Quite comparable effects on pH are seen when mannitol administration have made diuresis to occur in either humans or experimental animals [50].

Tests for glucose in urine have done more frequently than any other single chemical or biological urinary measurement [51]. Such tests have done with great frequency in the procedures of screening healthy person for the identification of asymptomatic disease, as a part of diagnostic workup in the recognition of diabetes, or for differential diagnosis in resolving the problems of the crises of diabetes. Eventually, tests of sugar in the urine provide an important monitoring mechanism for diabetic patients to assess the effectiveness of their control by medication or by diet [52]. This gleaning information of the diabetic is utilized by the physician in the regulation of the disease.

Within recent years, there has been marked an increment in the use of urine sugar tests which reflects the expansion on screening tests and patient monitoring of the treatment of their disorder. It is frequently known that normal urine does not have glucose, but need to qualify since there is a minute quantity of glucose in all normal urine [53]. However, both specific tests of glucose which employ enzymes and non-specific tests for reducing sugar have adjusted sensitivity so the normal urine gives a negative reaction [54].

The concentration of glucose in the blood is usually between 65 and 80 mg / 100 ml during the fasting time [55]. This concentration does not decrease according to the mechanism of the formation of glomerular. The rate of reabsorbtion is actually a little lower in glucose than in water. It means that the final urine contains a very minute amount of glucose. Schersten and and Fritz [56] have indicated that normal urine usually contains more than 2 mg / 100 ml than up to 20 mg / 100 ml of glucose concentration.

The concentration of glucose in the glomerular filtrate is increased correspondingly when the blood sugar is concentrated on more than 180 – 200 mg [57]. Instancelly, the relative amount of water reabsorption greatly exceeds the amount of glucose reabsorption and the capability of the tubule cells to phosphorylate so that lead to increase a glucose concentration in the urine which is ranging from 100 mg / 100 ml to more than 10,000 mg / 100 ml [58]. Above of this level is known as the renal threshold.

The kidney is reabsorbed these kinds of sugars, galactose, fructose, xylose, lactose, sucrose, mannose and others in the same way of glucose [59]. These sugars will appear in the glomerular filtrate, when they are exist in the blood and will not be as rapidly reabsorbed as the water of the filtrate. Accordingly, there is a quantity very much greater than in blood in the final urine. No more sugars rather than glucose appear to have a renal threshold [60].

A test for the presence or absence of protein in the urine is one of the most frequently performed procedures in routine urinalysis [61]. Before more than one century, clinical tests were based on precipitation phenomena involving the coagulation of protein by heat and various chemical agents, including concentrated nitric acid, trichloroaceetic acid and sulfosalicylic acid [62]. The Merck Index, 5th edition [63] listed about 33 different tests for protein (albumin) in urine. In 1957, the dip-and-read colorimetric test in most parts of the world [64]. The colorimetric dip-and-read test provides a satisfactory degree of specificity and also gives a semiquantitation of the exist amount of protein. There are also tests which identify the presence of various specific proteins or groups of protein in the urine is a screening procedure which is applied to all patients and utilized for evaluation studies with healthy subjects [65].

The mechanism of protein to be into urine is not completely obvious. Kark *et al.* [66] suggested five possibilities pathways of entry of protein into urine that were passage of protein across the glomerular membrane, disturbance of the normal tubular resorption of protein, abnormal secretion of protein from the plasma by the tubular cells, loss of plasma proteins from the lymphatics of renal papillae and abnormal secretion of genitourinary tract proteins.

More than one of these mechanism might be explained the excretion of protein in urine. Rennie [67] has reviewed and discussed the subject of proteinuria and has indicated that the evidence is quite overwhelming in support of the concept that protein leaks from the serum through the glomerulus and subsequently reabsorbed in the tubule. Ordinarily, there is a clear-cut relation between the size of the protein molecule and its rate of clearance.

Thus, by all the mechanism and physical properties of urine, it is obviously an amazingly complex entity which has many advantages to study. Urine has much information of a varied nature to contribute the measurement of many chemical and physical parameters. The analysis of urine can provide important information of the body functions and health.

1.2 Helium-Neon Laser

The laser tube in a He-Ne laser consists of a long a discharge tube filled with the active medium that is a mixture of about 10 parts of helium to one part of neon [68]. The gas mixture of helium and neon forms the lasing medium and this mixture is enclosed between a set of mirrors forming a resonant cavity consists of a plane, high-reflecting mirror at one end of the laser tube, and a concave output coupler mirror of approximately 1% transmission at the other end.

The red He-Ne laser wavelength is usually reported as 632.816 nm [69]. This is in fact the wavelength in air, and corresponds to a vacuum wavelength of 632.991 nm. The precise operating wavelength lies within about 0.002 nm of this value, and fluctuates within this range due to thermal expansion of the cavity [70]. The light emission from gas lasers as compared to that from solid state lasers is found to be more directional and much more monochromatic [71]. This is due to the various imperfections present in the solids and also the heating caused by the flash lamp. Even though solid state diode lasers can now provide red laser light beams with intensities comparable to those obtained with He-Ne lasers, it is anticipated that the He-Ne laser will remain a common component in scientific and technical instrumentation in the foreseeable future [72].

In this research, 0.95 mW He-Ne laser of wavelength 632.8 nm is used to find the correlations of urine parameters and flux variations of human urine. Significant relationships between urine parameters is studied by obtaining patterns and statistically tests using SigmaStat 3.1. Urine parameters have studied on flux variations using He-Ne laser and Encircled Flux Analysis System (EFAS). The functions of He-Ne laser in medical fields can be wider by using it as a tool to study the changes that happened in the urine in corporation with EFAS. It can contribute to medical investigation for diagnosis or theraphy planning. This thesis study and discuss the flux peaks and total flux patterns of males and females in the age groups ranged 20-29 years to 70-79 years old.

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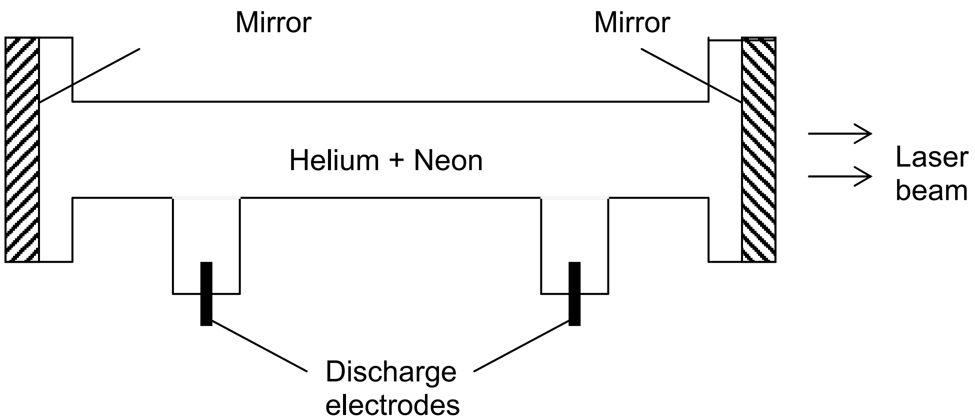

Figure 1.2. Schematic of helium-neon laser [73].

1.2.1 Flux of Laser

. The flux of a quantity is defined as the rate of flow of energy through a given surface [74]. Flux has been used in laser ablation which is the process of removing material from a solid (or occasionally liquid) surface by irradiating it with a laser beam [75]. It also been used to study a characteristic pattern of laser Doppler flux (LDF) of patients with venous ulcer before and after ulcer healing [76]. The flux became a significant parameter in the study on laser needle acupuncture [77]. The changes in human urine when aging also has been studied by finding patterns and correlations in flux variations [78]. In this research, the flux peak and total flux are been used as a significant parameters. The flux peak is the value of the peak flux in counts while the total flux is the value of the total flux in the beam area.

1.3 Objectives of the Research

It is most likely that one of the trends of the future will be to utilize urine as a means of reinforcing the concept that a state of health prevails [79]. The correlation of more information of the urine certainly must be effectively utilized. There also a significant progress in the evolution of new as well as improved chemical and physical test systems which are important in urine study.

Hence, the focus of this research are to find out the correlations between urine parameters such as urine pH, urine specific gravity, urine protein, urine glucose and age. This research also aim to obtain the range of flux levels of patients urines and to observe the 2D contour and 3D profile of the patients according to gender.

1.4 Outline of Thesis

As an introduction to the background theory, Chapter 1 contains a summary of human urine and Helium-Neon laser which are the fundamental knowledges in this study. In Chapter 2, attention is paid to the explanation of the urine samples, instrumentation and experimental set-up used in this research. Chapter 3 discusses the obtained results and statistical analysis techniques used to study the samples whereas Chapter 4 contains the 2D countour and 3D profile images. Finally, Chapter 5 summarises the conclusions of this thesis, and recommendations for future work.

1.5 Literature Review

This research used a new technique which not being done yet by other researchers. Because of that, there were no published journals or references that have used laser in their urine research. There were some researchers that used the conventional methods to study urine test and correlation with disease such as about urinary tract infection [80]. Consequently, urine is widely studied as an aid in diagnosis and monitoring the course of treatment of disease [81]. The correlation between urine parameters has been studied by other researcher such as P. Tabouleta *et al* [82] that doing research on the correlation between urine ketones (acetoacetate) and capillary blood ketones (3-beta-hydroxybutyrate) in hyperglycaemic patients . There are also study on correlation between urine and the patients that under the influence of drugs of abuse [83] .

In the medical applications, He-Ne laser has been widely used in various thrust. It is expected to be used extensively in the treatment of cancer [84]. He-Ne laser has been used for clinical PhotoDynamic Therapy in China [85]. Helium-Neon laser also has became therapeutic tool to reduce the risk of acute myocardial infarction in the patients [86] and the treatment of diabetic patients [87].

There are two main methods to do the urine test in the hospitals and clinics in Malaysia, there are by using reagent dipstick which will change colour when in contacts with urine and laboratory test which will use machine such as Urisys 1800 that being used in Penang Hospital. The using of this laser technique to do the urine test is better than other conventional techniques because of more faster, cleaner and easier procedures. This technique is also a better technique because it can give the reading in flux parameters such as flux peak which is the peak counts in the beam area and total flux which is the amount of flux in the beam area.

Chapter 2 : Materials And Methods

2.1 Urine samples

Human urines were kindly provided by Penang Hospital, Malaysia. A total of 160 urine samples from 82 males and 78 females were used in this research (Figure 2.1). Urine samples were obtained from patients range from 20 to 79 years old. 5 plastic containers of urine were obtained in 5 days every week from Monday to Friday. The urine were received at 9.00 am in the morning and by 11.00 am, the process of labelling were done to the urine samples in the lab. The samples were labelled with the coding systems according to gender, disease and age.

2.2 Instrumentation

The 0.95 mW He-Ne laser has been used as a power source and Encircled Flux Analysis System (EFAS) Model 8350, Photon Inc. as a flux detector (Figure 2.2). He-Ne laser (λ=632.8 nm) has been choosen as a power source because of its properties operation which are suitable for this research work and non-destruction tests can be done that it is very important for the samples. The major system hardware components were Photon Model 2320 BeamPro$_{filer}$ CCD camera with built-in variable optical attenuator, 60X objective lens, Photon Magnifying Objective Lens Mount for mounting the camera and lens, high precision XYZ translation stage with rail mount, Photon Model 3180

Controller, Photon $BeamPro_{filer}$ Image Capture Card and Encircled Flux Analysis Software for Windows 95/98/ME and Windows NT4.0/2000 Professional. A statistical software named SigmaStat 3.1 has been used to perform effective and accurate statistical analysis and interpretation to the results.

2.3 Experimental methods

The research was carried out in the lab after the proccess of labelling and coding have been done to the samples. A 5 ml catridge was used to draw the urine from the plastic container and 0.2 ml drops of each urine sample was injected on 7.5 cm x 2.5 cm slide. The slide was placed between power source and the detector, 12 cm away from He-Ne laser and 1 cm from EFAS which was connected to the computer that installed with its software (Figure 2.3). Then, the He-Ne laser was switched on and a set of 10 readings were obtained for each urine sample. In order to avoid interference from the environment light, the whole set-up was placed in a box which acts as a research platform (Figure 2.4). After the study have been completely done, all of the urine samples in the plastic container was discarded in the black plastic into the dustbin.

The values of the flux peak and total flux and also the 2D contour and 3D profile will be show on the computer screen. The graph are plotted from the obtained values. The values also been analized statistically using SigmaStat 3.1. To obvious more clearly the patterns of 2D contour and 3D profile, the freeform line is used by joining the highest beam intensity on the beam area.

Figure 2.1. Urine samples

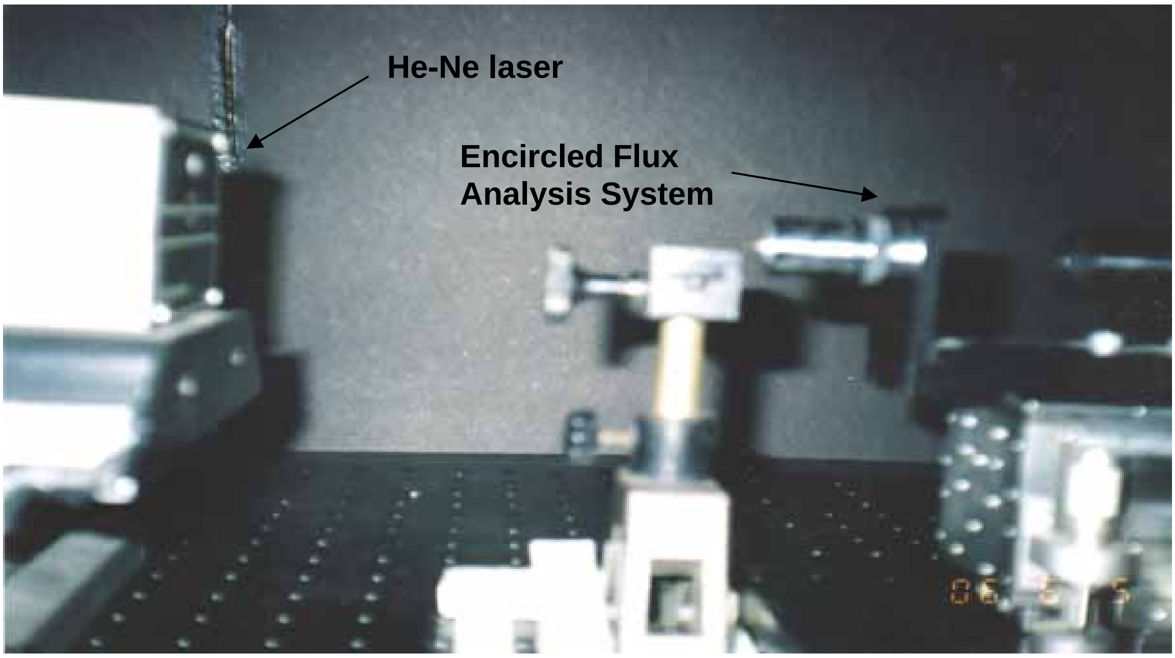

Figure 2.2. He-Ne laser (0.95 mW) and Encircled Flux Analysis System (EFAS) Model 8350, Photon Inc.

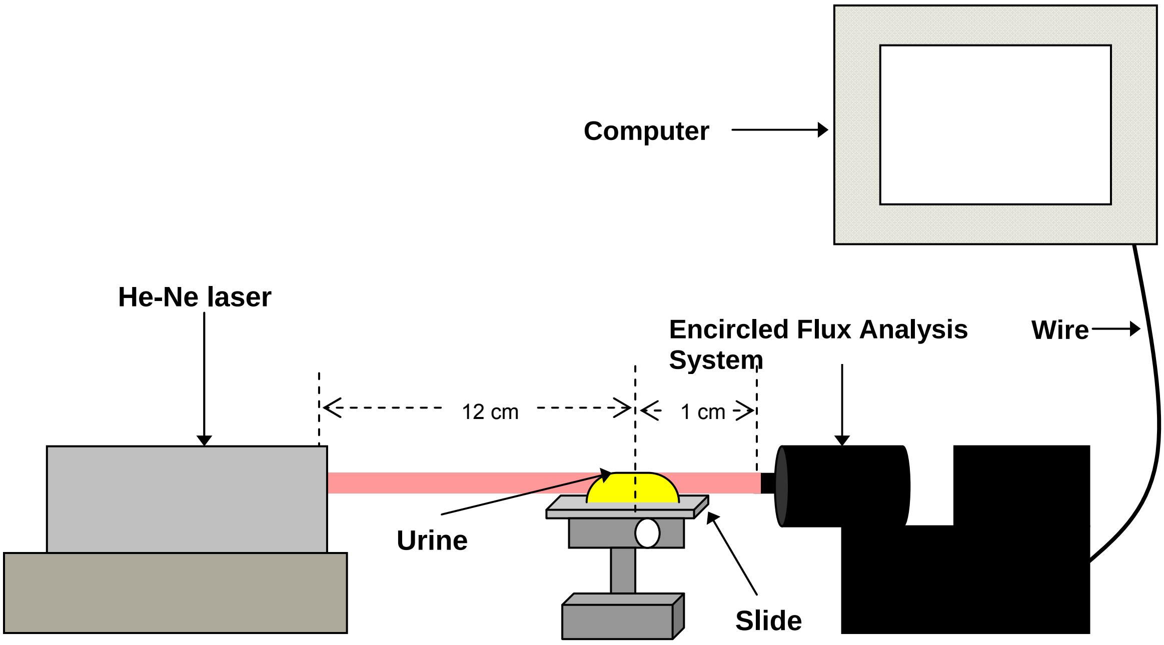

Figure 2.3. Schematic diagram of the experimental set-up.

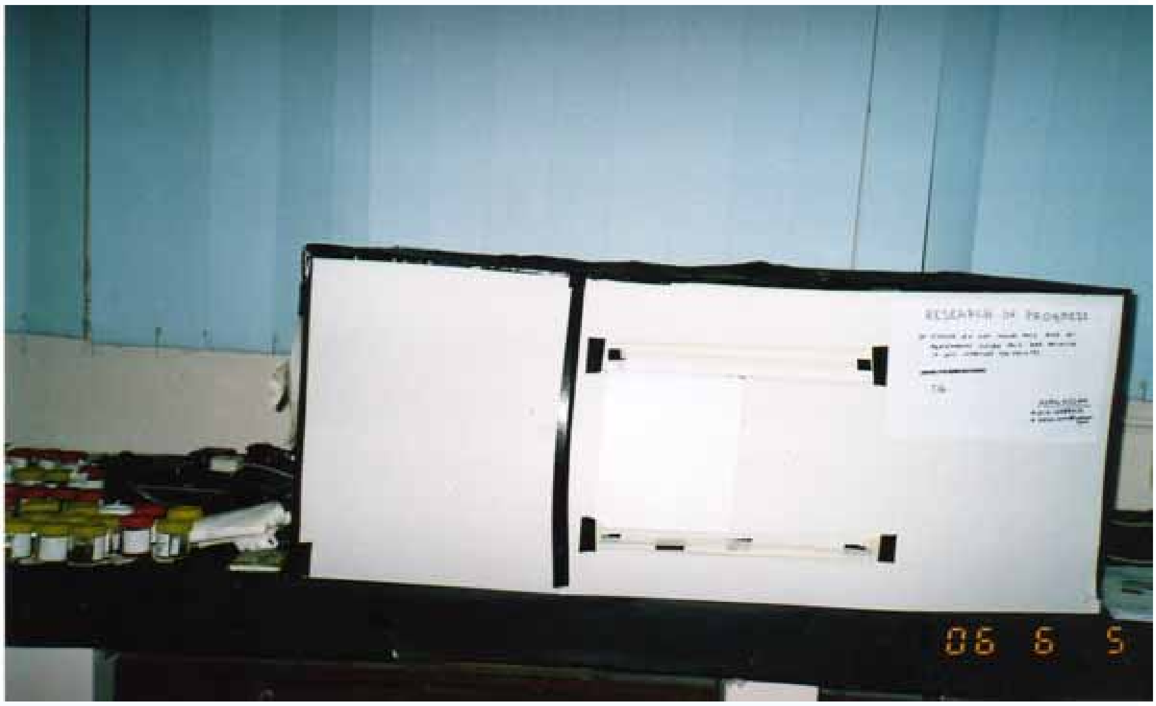

Figure 2.4. The box that was used to avoid interference from the environment light and acts a research platform.